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NUMBER AND DISTRIBUTION OF INTERSTITIAL CELLS OF CAJAL IN HUMAN GALLBLADDER

Abstract: Based on the review of current literature and on their own studies authors postulate that decreased number of interstitial cells in the wall of gallbladder may be pathognomonic for gallstone disease (cholelithiasis).

Key words: interstitial cells of Cajal, human gallbladder.

INTRODUCTION

It was Santiago Ramon y Cajal who described for the first time in the world interstitial cells [1, 2]. Following his opinion these cells played role of separate nervous cells, relatively rich within intestinal smooth muscle, where they united with autonomic nerve bundles forming dense. The cells varied according to shape. Some were fusiform, triangular, sometimes multipolar (star-like). In Cajal's opinion they could play modulatory role in contraction of smooth myocytes of gastrointestinal system [2].

Interstitial cells were detected in many other tissues, always in the vicinity of smooth muscle cells. ICC were identified in gastrointestinal tract of many species, i.e. mice [3], rats [4], guinea pigs [5]. In humans ICC were found in the wall of alimentary tract [6], pancreas [7], in the muscle of atria and ventricles [8–10], vagina [11], mammary gland [12, 13], oviduct [14], ductus deferens [15], urinary tract [16–18], uterus [19], and in the blood vessels [20, 21].

Firstly ICC cells have not been found in the gallbladder [22]. However it was Xiaomin *et al.* who found in 2006 ICC cells in the mice gallbladder, and Lavoie *et al.* in 2007 of the guinea pig [23, 24]. In humans it was Ortiz-Hidalgo *et al.* who found ICC in the human gallbladder [25].

To conclude, the role of ICC is associated with: generation and modulation of slow waves within gastrointestinal tract, facilitation of propagation of slow waves, their coordination and mediation in transmission of excitatory or inhibitory stimu-

lation (neurotransmission) between autonomic nervous system and muscular cells.

In certain diseases of the alimentary system the number of interstitial cells is significantly decreased. The direct mechanism of regression of Cajal cells is apoptosis [26], trans/de-differentiation [27, 28], while regeneration of ICC is associated with: proliferation of mature ICC [29], renovation of damaged cells and restoration from stem cells [30]. The newest reports say that mature ICC can still proliferate [31–33]. Kit signal path activated by kit-ligand is associated with control of survival and proliferation of ICC. We know also, playing similar role, other signal paths with neuronal originated nitric oxide [34], serotonin acting through 5-HT_{2B} receptor [35], interleukine-9 [36], insulin and insulin-like growth factor (IGF-1) [37], heme oxygenase (HO-1) [38]. 5-HT_{2B} receptor is responsible for maintenance of network of ICC [39]. Its activation begins proliferation of ICC. Knock-out mice which did not have that mutation of locus W, coding c-kit protein in mice (W^v/W) and rats Ws/Ws) leads to decrease of interstitial cells of Cajal in myenteric plexus of small intestine and stomach, what causes regression of slow waves [40]. Similar result was achieved by application of antibodies which blocked c-kit receptor in mice. Decrease of number or malfunction of interstitial cells is associated with numerous diseases of gastrointestinal system, i.e. idiopathic perforation of stomach, Hirschprung disease, gastroparesis and many others [41]. Changes according to number and function of interstitial cells which integrate motor activity of alimentary tract may play role also in gallstone disease [42–46]. Mutation in gene coding kit receptor leads to transformation of interstitial cells and gastrointestinal stromal tumors (GIST) development [47].

MATERIAL AND METHODS

The studies were carried out on 55 patients (19 males and 39 females, aged 27–80) operated in the 1st Department of General Surgery JU MC in 2010. The study was approved by Ethical Committee of Jagiellonian University (KBET/30/B/2010) according to Helsinki Declaration.

30 of patients were operated due to symptomatic cholelithiasis — using laparoscopic cholecystectomy. Before surgery in all patients normal level of bilirubin was confirmed. Control was based on 25 patients qualified to planned surgery because of tumor of pancreatic head, with no pre- and postoperative signs of cholelithiasis. Normal level of bilirubin was also required.

Obtained gallbladders were sectioned longitudinally and washed in phosphate buffer saline (PBS), next they were placed in 4% solution of buffered paraformaldehyde. The staining last 24 hrs. in room temperature. Next the material was washed in PBS. The gallbladders were examined macroscopically. An excision from each specimen was made — a band 4–5 mm wide, including the fundus, the body and the neck. Excisions obtained were properly marked with the ink. Next

the material was dehydrated in rising concentrations of alcohol, then washed in xylene. After immersing in paraffin at the temperature 56°C, tissue blocks were made. Next paraffin blocks were cut on microtome (Jung BioCut 2035, Leica, Wetzlar, Germany) into slices 6μm thick. The slices were deparaffinized in xylene (for immunostaining) and next placed in PBS solution. As control we used paraffin blocks of ileum obtained from collection of Department of Histology, Jagiellonian University, Medical College. To visualize the interstitial cells of Cajal (ICC) we used the method which was described in [46].

RESULTS

Pathologic evaluation of the gallbladders studied showed signs of chronic inflammation (chronic cholecystitis) in the wall. Immunohistochemical methods used (indirect double immunofluorescence staining) allowed identification of cells which revealed positive reaction with c-kit antibody. Among these cells we could find both ICC and numerous mastocytes. Application of antibodies against mast tryptase (normally found within mastocytes) enables differentiation between mast cells and ICC. Under the microscope we could observe c-kit immunopositive cells (red) and positive to tryptase (green), more — bluish nuclei, which one could see using specific filters. For statistical analysis ICC were: c-kit immunopositive, tryptase-immunonegative, presenting nuclei (to distinguish them from incidental non-nuclear structures). ICC were visible in the entire area of the band including fundus, body and neck of the gallbladder. They were found practically only within muscular layer, where they paralleled smooth muscle fibers. Microscopic analysis of specimens proved the following distribution of interstitial cells: intramuscular (ICC-IM), between the fibers of smooth muscle; interbundle — ICC-IB — within the connective tissue separating bundles of smooth muscle fibers. ICC existed usually as single cells, sometimes in groups of 2–3 cells, but we did not find networks of these cells.

Interstitial cells possessed characteristic elongated, fusiform shape — their length varied from 40–60 μm, in some specimens we could observe their processes. Besides, in some specimens we have observed few c-kit immunopositive cells (being simultaneously tryptase-negative), roundish in shape, which following the configuration of immunologic markers were assessed as ICC. C-kit immunopositive mast-cellshad usually roundish or oval shape, centrally placed nucleus, what enabled their differentiation from ICC. In control the average number of the interstitial cells was 7.06 per one visual field. We did not analyze differences between certain parts of gallbladder regarding the content of ICC. The average number of ICC obtained from bands of gallbladders of patients with cholelithiasis was 3,35/one visual fiend and it was twice lower in comparison to the control. The difference was statistically significant ($p < 0{,}0001$). We did not observe diffe-

rences in morphology and size of ICC which came from group of patients with cholelithiasis and control, estimated under the light microscope. We did not find also differences in distribution of ICC. We did not state dependence between the number of ICC and age.

DISCUSSION

Cholelithiasis is nowadays a major problem of modern medicine. Operations on gallbladder (cholecystectomies) due to gallstone disease are the most common procedures carried out in surgical departments. Following current trends cholelithiasis is a polietiologic disease. Pathogenesis of gallstone disease is a subject of many articles and according to widely accepted theories it is caused mainly by disturbances of lipid composition of bile and bile retention resulted from weakening of motor activity of gallbladder [48–51].

Understanding of physiology of smooth muscles of gastrointestinal system throughout last decades has significantly improved, mostly thank to studies on population of ICC. Discovery of immunoreaction of ICC with antibody against c-kit antigen opened new era in methodology of identification of ICC. ICC are present in numerous organs, but their morphology and function was best recognized in alimentary tract. It was established that these cells play role of pacemakers and their dysfunction may become a basis for motor disturbances of gastrointestinal system. This is why it seems that role of ICC is associated with: generation and modulation of slow waves in gastrointestinal tract, facilitation and propagation (promotion) of slow waves and their coordination, transition of transmission of excitatory or inhibitory stimuli (neurotransmission) between the autonomic nervous system and muscular cells. Knowledge on dependence of ICC and c-kit receptor for their normal development and maturation was a milestone, because it began wide application of immunohistochemical technics for identification of ICC [52].

ICC in the biliary system are not so long known. For the first time it was Ortiz-Hidalgo *et al.* in 2000 who presented stromal tumor of gallbladder containing cells phenotypically similar to ICC [25]. In 2007 Hinescu *et al.* described for the first time ICC cells in human gallbladder removed for non-neoplasmatic reasons [26]. Ahmadi *et al.* found ICC cells in extrahepatic biliary tracts in 2009 [53]. There's a lack of further reports on it in current literature, nor information on the distribution of ICC in the subsequent layers of wall of human gallbladder. In present study a number of ICC cells were identifies in the wall of gallbladder using indirect double immunofluorescence with antibodies against c-kit receptor and mast-tryptase.

Hinescu *et al.* [26] to identify ICC cells used immunoperoxydase staining with antibody anti-c-kit/CD 117. However we must remember that such staining allows dying of all c-kit positive cells, both ICC and mast cells. Sometimes it is

not possible to differentiate these two lines of cells using morphological features seen under the microscope. From another hand however Ahmadi *et al.* used the same method which we used [53].

In our study we found significant decrease of number of ICC cells in the wall of gallbladders of patients suffering from gallstone disease in comparison to the control. Such decrease may influence motor activity of the gallbladder. Numerous articles indicate different dysfunctions of the gallstone diseased patients [54–60]. We can conclude that such change leads to problems in bile flow, what in consequence causes development and deposition of concretions in the lumen of the gallbladder. Considering the role of ICC cells for the motor activity of the gastrointestinal system, the decreased number of ICC in the wall of the gallbladder correlates with motor dysfunction of the organ and reduction of number of interstitial cells is an important pathognomonic factor in biliary lithiasis.

CONFLICT OF INTERESTS

None declared.

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